

LISTING OF  
~~AMENDMENTS TO THE CLAIMS~~

1-52. (Canceled)

53. (Previously Presented) An infectious chimeric parainfluenza virus (PIV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a partial or complete human parainfluenza virus 3 (HPIV3) background genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) encoding a complete open reading frame or one or more antigenic determinant(s) selected from the group consisting of:

- an HN glycoprotein of HPIV 1,
- an HN glycoprotein of HPIV2,
- an F glycoprotein of HPIV1 and
- an F glycoprotein of HPIV2,

said heterologous gene(s) or gene segment(s) being operably linked to regulatory sequences operable in said HPIV3 genome or antigenome, to form a chimeric parainfluenza virus (PIV) genome or antigenome;

said partial or complete PIV background genome or antigenome comprising a polynucleotide encoding a wild-type L protein of the background PIV;

said infectious chimeric PIV being attenuated for replication at least 10-fold in the respiratory tract of a primate host infected with said chimeric PIV.

54. (Previously Presented) An infectious chimeric parainfluenza virus (PIV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a partial or complete human parainfluenza virus 3(HPIV3) background genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) encoding a complete open reading frame or one or more antigenic determinant(s) of HN and/or F glycoproteins of HPIV1 and/or HPIV2, said heterologous gene(s) or gene segment(s) being operably linked to regulatory sequences operable in said HPIV3 genome or antigenome, to form a chimeric PIV genome or antigenome;

said heterologous gene(s) or genome segment(s) being inserted into the HPIV3 background genome at one or more site(s) selected from the group consisting of a site between the P and M open reading frames, a site between the N and P open reading frames, a site between the HN and L open reading frames.

55. (Previously Presented) The infectious chimeric PIV of claim 54, in which the heterologous gene(s) or genome segment is inserted between the HN and L open reading frames.

56. (Previously Presented) An infectious parainfluenza virus parainfluenza virus (PIV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a partial or complete human parainfluenza virus (HPIV) background genome or antigenome combined with one or more heterologous gene(s) or genome segment(s), said heterologous gene(s) or genome segment(s) being inserted at one or more site(s) selected from the group consisting of a site between the P and M open reading frames, a site between the N and P open reading frames, a site between the HN and L open reading frames,

which is attenuated *in vivo* at least 10-fold compared to replication of the corresponding wild-type PIV.

57. (Previously Presented) The infectious PIV of claim 56, in which the heterologous gene(s) or genome segment(s) are inserted at a site between the HN and L open reading frames.

58. (Previously Presented) The infectious PIV of claim 56, in which the heterologous gene(s) or genome segment(s) comprise a gene start sequence and a gene end sequence of the background HPIV virus genome or antigenome.

59. (Previously Presented) The infectious PIV of claim 56, in which the heterologous gene(s) or genome segment(s) are inserted into the non-coding region of the HN gene of the background HPIV genome or antigenome.

60. (Previously Presented) The infectious PIV of claim 56, in which the heterologous gene(s) or genome segment(s) is a polynucleotide that does not encode a protein.

61. (Previously Presented) The infectious PIV of claim 56, in which the heterologous gene(s) or genome segment(s) have a length of at least 996 nucleotides.

62. (Previously Presented) The infectious PIV of claim 60, in which the polynucleotide has a length of at least 996 nucleotides.

63. (Previously Presented) The infectious PIV of claim 62, in which the polynucleotide comprises a gene start sequence and a gene end sequence of the background HPIV virus genome or antigenome.

64. (Previously Presented) The infectious PIV of claim 62, in which the polynucleotide sequence is inserted into a 3' untranslated region of a PIV gene.

65. (Previously Presented) The infectious PIV of claim 56, in which the heterologous gene(s) or genome segment(s) have a length of at least 3000 nucleotides.

66. (Previously Presented) The infectious PIV of claim 56, in which the heterologous gene(s) or genome segment(s) are obtained from the measles HA gene.

67. (Previously Presented) An infectious chimeric parainfluenza virus (PIV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a partial or complete human parainfluenza virus 3 (HPIV3) background genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) encoding a complete open reading frame or one or more antigenic determinant(s) of HN and/or F glycoproteins of HPIV 1 and/or HPIV2, said heterologous gene(s) or gene segment(s) being operably linked to regulatory sequences operable in said HPIV3 genome or antigenome, to form a chimeric PIV genome or antigenome;

said partial or complete HPIV3 background genome or antigenome including a mutation encoding a substitution of amino acid 456 of the L protein by another amino acid.

68. (Previously Presented) An immunogenic composition comprising an immunologically effective amount of the infectious chimeric PIV of any one of claims 53-67 and a physiologically acceptable carrier.

69. (Previously Presented) An isolated polynucleotide encoding a partial or complete human parainfluenza virus 3 (HPIV3) background genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) encoding a complete open reading frame or one or more antigenic determinant(s) selected from the group consisting of:

- an HN glycoprotein of HPIV 1,
- an HN glycoprotein of HPIV2,
- an F glycoprotein of HPIV1 and
- an F glycoprotein of HPIV2,

said heterologous gene(s) or gene segment(s) being operably linked to regulatory sequences operable in said HPIV3 genome or antigenome, to form a chimeric parainfluenza virus (PIV) genome or antigenome;

said partial or complete PIV background genome or antigenome comprising a polynucleotide encoding a wild-type L protein of the background PIV.

70. (Previously Presented) An isolated polynucleotide comprising a polynucleotide sequence encoding a partial or complete human parainfluenza virus 3(HPIV3) background genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) encoding a complete open reading frame or one or more antigenic determinant(s) of HN and/or F glycoproteins of HPIV1 and/or HPIV2, said heterologous gene(s) or gene segment(s) being operably linked to regulatory sequences operable in said HPIV3 genome or antigenome, to form a chimeric PIV genome or antigenome;

said heterologous gene(s) or genome segment(s) being inserted into the HPIV3 background genome at one or more site(s) selected from the group consisting of a site between

the P and M open reading frames, a site between the N and P open reading frames, a site between the HN and L open reading frames.

71. (Previously Presented) The isolated polynucleotide of claim 70, in which the heterologous gene(s) or genome segment is inserted between the HN and L open reading frames.

72. (Previously Presented) An isolated polynucleotide comprising a polynucleotide sequence encoding a partial or complete human parainfluenza virus (HPIV) background genome or antigenome combined with one or more heterologous gene(s) or genome segment(s), said heterologous gene(s) or genome segment(s) being inserted at one or more site(s) selected from the group consisting of a site between the P and M open reading frames, a site between the N and P open reading frames, a site between the HN and L open reading frames.

73. (Previously Presented) The isolated polynucleotide of claim 72, in which the heterologous gene(s) or genome segment(s) are inserted at a site between the HN and L open reading frames.

74. (Previously Presented) The isolated polynucleotide of claim 72, in which the heterologous gene(s) or genome segment(s) comprise a gene start sequence and a gene end sequence of the background HPIV virus genome or antigenome.

75. (Previously Presented) The isolated polynucleotide of claim 72, in which the heterologous gene(s) or genome segment(s) are inserted into the non-coding region of the HN gene of the background HPIV genome or antigenome.

76. (Previously Presented) The isolated polynucleotide of claim 72, in which the heterologous gene(s) or genome segment(s) is a polynucleotide that does not encode a protein.

77. (Previously Presented) The isolated polynucleotide of claim 72, in which the heterologous gene(s) or genome segment(s) have a length of at least 996 nucleotides.

78. (Previously Presented) The isolated polynucleotide of claim 76, in which the polynucleotide has a length of at least 996 nucleotides.

79. (Previously Presented) The isolated polynucleotide of claim 78, in which the polynucleotide comprises a gene start sequence and a gene end sequence of the background HPIV virus genome or antigenome.

80. (Previously Presented) The isolated polynucleotide of claim 78, in which the polynucleotide sequence is inserted into a 3' untranslated region of a PIV gene.

81. (Previously Presented) The isolated polynucleotide of claim 72, in which the heterologous gene(s) or genome segment(s) have a length of at least 3000 nucleotides.

82. (Previously Presented) The isolated polynucleotide of claim 72, in which the heterologous gene(s) or genome segment(s) are obtained from the measles HA gene.

83. (Previously Presented) An isolated polynucleotide comprising a polynucleotide sequence encoding a partial or complete human parainfluenza virus 3 (HPIV3) background genome or antigenome combined with one or more heterologous gene(s) or genome segment(s) encoding a complete open reading frame or one or more antigenic determinant(s) of HN and/or F glycoproteins of HPIV 1 and/or HPIV2, said heterologous gene(s) or gene segment(s) being operably linked to regulatory sequences operable in said HPIV3 genome or antigenome, to form a chimeric PIV genome or antigenome;

said partial or complete HPIV3 background genome or antigenome including a mutation encoding a substitution of amino acid 456 of the L protein by another amino acid.

84. (Previously Presented) An expression vector comprising in operable linkage:

- i) a promoter operable in a mammalian cell or *in vitro*;
- ii) the polynucleotide of any one of claims 69-83; and
- iii) a transcription terminator sequence operable in a mammalian cell or *in vitro*.

85. (Previously Presented) A method for making an infectious PIV from one or more isolated polynucleotides comprising:

expressing in a cell or cell-free lysate an expression vector comprising the isolated polynucleotide of any one of claims 69, 70, 72 and 83, and N, P and L proteins of a PIV;

wherein one or more of said N, P and L proteins of a PIV can be encoded by the isolated polynucleotide or by one or more separate expression vectors.